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SOME OBSERVATIONS ON A PHOTOGENIC MICRO-ORGANISM, *PSEUDOMONAS LUCIFERA* MOLISCH.

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Some years ago Dr. R. E. B. McKenney published in the Proceedings of the Biological Society a very interesting paper on luminous bacteria (Proc. Biol. Soc. Wash., 1902, Vol. 15, pp. 213–234). The form which serves as the basis for what is to follow has been isolated since Dr. McKenney's paper was published, and appears to present some points of possible interest. This organism is *Pseudomonas lucifera* Molisch, isolated by Prof. Hans Molisch, of the Plant Physiology Institute of the University of Vienna, and it is through the courtesy of Professor Molisch that the parent culture for this work was obtained. The organism was isolated from sea-water, and is claimed by the discoverer to give the brightest light of any bacterial form so far isolated.

Like most other luminous micro-organisms, *Ps. lucifera* will grow on the ordinary culture media, under aerobic conditions, but for luminescence there must be present 2.5 to 3.0 per cent of sodium chloride, or some one of certain other mineral salts. The use of media made from fish-meat is unnecessary. The light given by ordinary bouillon-gelatin-salt cultures is a soft and beautiful green, which after the eyes become accustomed to it, appears of considerable intensity. A veritable "living lamp," as Dubois has called it, may be made by coating the inside of a sterile flask with the bouillon-gelatin-salt medium, and then inoculating the surface of the gelatin with a liquid culture. Such a lamp will continue to give light for about a week, though the light

gradually weakens, owing to the drying of the medium, and finally dies out; the maximum intensity of light is about 48 hours after inoculation.

Agar cultures appear to grow about as well as gelatin cultures but luminesce weakly or not at all, even in the presence of the proper amount of mineral salts. Liquid media of very simple composition serve for the growth of the organism; the simplest medium serving for luminosity is a 3.0 per cent solution of salt, containing 1.0 per cent of asparagin.* This medium is of course not the best; cultures in it are short-lived, and glow only feebly; a better medium is—

Sodium chloride	2.5%
Magnesium chloride	.5
Peptone	1.0
Asparagin	1.0
Glycerin	.5

Larger amounts of peptone and asparagin act adversely to growth; the organism is apparently sensitive to "over-feeding." It will grow and luminesce in milk containing 3.0 per cent of common salt; the light on the surface is quite bright. As a rule, however, the light from luminous cultures is much less intense than that from gelatin, though when shaken gently, the light perceptibly increases in intensity. The mineral salt present may be any one of several, but the brightest cultures have been obtained by the use of 2.5 per cent of sodium nitrate, and 0.5 per cent of magnesium chloride. All media must be faintly alkaline to litmus.

The spectrum of the light emitted by a gelatin culture is of very limited range, extending from the yellow-orange to the indigo, with a decided maximum intensity in the green. This spectrum is of less extent than that of our local fireflies (*Photinus pyralis*, *Photuris pennsylvanica*, etc.) although the appearance of the light to the eye does not differ so very much from that of the last-mentioned Lampyrid. In liquid cultures the light appears to the eye to be more whitish, though the range of the spectrum is the same; probably the maximum intensity is shifted to a different point.

* In this connection it is of interest to note that Wood (Journ. Amer. Med. Assn., 1911, Vol. 56, pp. 1094-6), has recently recommended "normal" (physiologic) salt solution as an emergency culture medium for many bacteria.

Forsyth (Nature, 1910, Vol. 43, p. 7) has reported the discovery, by spectrophotography, of ultra-violet rays in the light of *Photobacterium phosphorescens* Fischer. This seems very remarkable, especially in view of the fact that ultra-violet light is used as a bactericide, and the further fact that previous photographs of the spectrum of the light of these organisms had failed to show the presence of such rays. In this connection solutions and crystals of para-amino-ortho-sulpho-benzoic acid* have been exposed to the light from large cultures of *Ps. lucifera*, and have failed to show the least trace of fluorescence, although this substance is used to detect ultra-violet radiation by means of its fluorescence. It seems very unlikely that this organism emits ultra-violet radiation.

Luminous cultures of *Ps. lucifera* on the usual culture media do not appreciably affect a charged electroscope. For the conduct of the experiments leading to this conclusion, I am indebted to Drs. F. W. Clarke and R. C. Wells, of the Geological Survey.

In its chemical conduct this micro-organism exhibits only certain slight similarities to the firefly. Professor Kastle and the author have shown that the luminous tissue of the Lampyridae which has been dried in the absence of oxygen—preferably in a hydrogen vacuum—and sealed in hydrogen, will exhibit luminosity when moistened, for at least thirteen months after preparation (Amer. Journal Physiol., 1910, Vol. 27, pp. 122–151, November), and since the above paper was written, specimens prepared at the same time have glowed on moistening after eighteen months; in fact there seems to be no good reason why they should deteriorate at all. The author has found that if commercial hydrogen peroxide solution is used in place of water for moistening the dry material, a much brighter light is obtained, whose spectrum is of about the same range as that of *Ps. lucifera*, though with its point of maximum intensity nearer the yellow. (Canadian Entomologist, November, 1910, Vol. 42, pp. 357–363.) Somewhat similar observations may be made upon this micro-organism. An open vessel filled with sulfuric acid was placed upon the bottom of a flask, the walls of which were coated with a luminous gelatin culture of *Ps. lucifera*, the flask filled with hydrogen, tightly stoppered, and set away. After two

* Kindly sent me by Prof. J. H. Kastle of the University of Virginia (See American Chemical Journal, January, 1911).

days the gelatin showed signs of being drier, and the culture did not glow. A small portion of the gelatin was removed by means of a spatula and placed in a Petri dish; it glowed feebly in the air, the glow being just perceptible in a dark room. A few cubic centimeters of 2.5 per cent hydrogen peroxide solution were then run upon the gelatin, when a number of bright points showed for an instant, after which no glow was visible. Attempts to completely dry liquid cultures, and to harden gelatin cultures in a hydrogen vacuum have so far been unsuccessful on account of the leakage of the apparatus. Nitrobenzol, which Dr. Kastle and the author found to be a powerful stimulus to activity on the part of the luminous tissue of the firefly, was without effect in stimulating the luminous activity of gelatin cultures of this organism whose luminosity was on the wane, and when added to liquid cultures promptly extinguished them. A solution of sodium nitrite added to a liquid culture of *Ps. lucifera* extinguished the light instantly. Both of these substances are germicides, and the results obtained are those which would naturally be foretold. The addition of a few drops of 1 : 10,000 adrenalin hydrochloride solution to 20 c. c. of a liquid luminous culture of this organism produced no immediate effect, but after 18 hours, the culture was apparently dead; adrenalin was found to be a powerful stimulant of photogenic activity in the firefly, when injected into the living insect. It would appear, therefore, that in the firefly, these exciters act upon the nervous system, and not directly upon the luminous tissue.

Oxygen under a maximum pressure of two or three atmospheres was applied to a liquid culture in a closed bulb; the light emitted became much stronger as long as the oxygen pressure was maintained; sudden release of the oxygen pressure was followed by a slow diminution of the intensity of the light. A liquid culture placed in a desiccator filled with hydrogen gave no light after about five minutes; it also failed to give light when treated with hydrogen peroxide at the end of three days, when it had dried out to the point of crystalization.

But little can be said as to the chemical processes by which these organisms produce light. The process is certainly one of oxidation, or at least one requiring the presence of oxygen, as is the case with the firefly. Probably the actual use made of

the oxygen is somewhat similar to use made of it in the luminous insects, except that the gas is taken by the cells directly from the air to which the organism is exposed, whereas in the firefly an intricate network of tracheæ supply the air for the process. But while the nature of the light emitted by these organisms, and some of their chemical properties suggests a general similarity of the photogenic processes in the two forms, it is by no means necessary to assume that it is identical. It may even prove that in organic chemistry we may have photophore groupings, as we now have fluorophore groups and chromophore groups, and that biologic oxidations producing very similar luminous manifestations may actually involve very different active substances. The fact that these organisms will luminesce in a medium consisting of water containing three per cent of salt and one per cent of asparagin—α-amino-succin amidic acid—

$$HOOC.CH_2.CHNH_2.CONH_2,$$

suggests that the process may really be less complicated than would at first seem.

So far as I know, *Ps. lucifera* is not pathogenic for man. It appears to be very small, and in common with most photogenic micro-organisms, it is rather delicate; it grows and luminesces best at 18–22° C.; 28 to 30° C. will kill the cultures.

The whole subject of the photogenic bacteria has been pretty thoroughly covered in Professor Molisch's book, Leuchtende Pflanzen (Jena, 1904), and a very exhaustive and interesting review of the entire subject of biophotogenesis,—regretably in German again,—has been published by Prof. Ernst Mangold of Greifswald, under the title Die Produktion von Licht, as the 2nd half of the third volume of Hans Winterstein's Handbuch der vergleichende Physiologie. (Jena, 1910.)

I have to thank Mr. Wm. Lindgren, of the Hygienic Laboratory, for propagation of this organism from the culture sent me, and for advice in handling the same.